Reference: *Biol. Bull.* **166**: 140–155. (February, 1984)

TRANSLOCATIVE FUNCTIONS OF THE ENIGMATIC ORGANS OF STARFISH—THE AXIAL ORGAN, HEMAL VESSELS, TIEDEMANN'S BODIES, AND RECTAL CAECA: AN AUTORADIOGRAPHIC STUDY

JOHN C. FERGUSON

Department of Biology, Eckerd College, Box 12560, St. Petersburg, Florida 33733

ABSTRACT

Starfish (*Echinaster graminicolus*) were fed C^{14}-labeled clams or liquid glucose-amino acid medium, and subsequently examined as autoradiographic sections. Ingested tracer was first incorporated into the cardiac stomach and lower-middle digestive gland. By 8 to 12 hours, increasing amounts were located throughout the axial organ, aboral hemal ring, radial hemal strand, and mesenteric hemal vessel. Subsequently (22 to 48 hours), label progressed into the genital hemal connectives and the gonads, as well as the connective tissue-hemal plexus of the tube feet. Clearly, hemal tissues, together with associated perihemal spaces, play major roles in the translocation of nutritive materials to the gonads and parts of the tube feet. These nutritive materials, however, may not come directly from the digestive system, which only has poorly developed hemal connections, but from coelomic sources.

Also noted was a gradual build-up of labeled material in the rectal caeca, suggesting that these organs remove nutrients from circulating digestive fluid before its evacuation through the anus or mouth. In animals fed liquid medium, tracer rapidly appeared in all parts of the water vascular system, showing that inflow of sea water does occur through the madreporite. Intense, rapid uptake by the Tiedemann's bodies revealed that they must filter a portion of this inflow, possibly producing coelomic fluid for body turgor and stomach inflation.

INTRODUCTION

The so-called "hemal system" of echinoderms has been the subject of much speculation but little experimental study (see review of Ferguson, 1982b). In starfish, this system consists of a peculiar "axial organ" lying in a coelomic space (axial sinus) adjacent to the stone canal, connecting strands (not really vessels) of "hemal" tissue located in the perihemal coelomic space above the radial nerve cord, and diminuitive aboral continuations to the several digestive organs and the gonads. Histologically, the system does not possess the normal characteristics of a circulatory system, and it appears to be unnecessary for that role since translocations of respiratory gases and nutrients are carried on by the coelomic fluids (Budington, 1942; Ferguson, 1964a, b).

Unexpected evidence that the hemal system does have a role in nutrient translocation, however, came forth in Ferguson's (1970) study on the incorporation of injected labeled amino acids by a starfish. Tracer accumulated in the radial hemal strand and in the connective tissue-hemal plexus of the tube feet. Unfortunately, this study did not examine other parts of the body, and thus, could only indicate a local translocative role between hemal tissue and the tube feet. More recent work, notably

Received 1 August 1983; accepted 14 November 1983.

that of Broertjes *et al.* (1979, 1980), also suggests an active transport role for the starfish hemal system, but the details are as yet unclear.

If the starfish hemal system has significant translocative functions, it should be possible to demonstrate movement of ingested nutrients into and through it. The present investigation was designed to do exactly that—to delineate autoradiographically the distribution of labeled food material in starfish tissues at various times after ingestion. Serendipitously, the study has shed light on not only the translocative role of the hemal organs, but also on the functions of several other enigmatic structures; most notably the rectal caeca, additional parts of the digestive system, the Tiedemann's bodies, the stone canal, and other components of the water vascular system.

Materials and Methods

Starfish were taken from the same population of Tampa Bay *Echinaster sp.* used by the author in numerous previous studies. These animals have recently been renamed *Echinaster graminicolus* (Campbell and Turner, 1984). They were obtained in early June, just after the spawning period, and were kept in the laboratory for two days before the start of the experiments. Two different methods were employed to induce them to ingest labeled nutrients whose subsequent distribution in the bodies could be followed.

The first set of experiments employed the recently confirmed (Ferguson, 1982a) ability of small bivalves (*Donax*) to rapidly take up dissolved nutrients from sea water. A group of these clams were placed for 12 hours in a dish containing 100 μCi C^{14}-labeled amino acids (synthetic algal protein hydrolysate) in filtered sea water. They were then thoroughly rinsed, broken open, and fed individually to starfish in a shallow container flushed with a strong stream of fresh sea water (to carry away any released dissolved label). Within 20 minutes all the animals had assumed a feeding posture, which they retained until removed or until digestion was completed 8 to 12 hours later. Individual starfish were removed at timed intervals (2, 4, 6, 8, 12, 24, and 48 hours after the beginning of feeding), immediately placed in ice cold (to slow decalcification) Bouin's fixative, and partially dissected. These animals were subsequently referred to as the "solid food group."

The second series of experiments used the observation (Ferguson, 1969) that *Echinaster* can be induced to directly ingest nutrient-rich sea water. A mixture of 375 mg glycine, 990 mg glucose, 100 μCi C^{14}-labeled amino acid mixture, and 10 mg chloramphenicol was dissolved in 1 l of filtered sea water. A group of starfish was then placed in this medium, and most of the animals soon were seen with everted stomachs in distinctive feeding postures. Specimens were removed, rinsed, and fixed in cold Bouin's after 3, 4.5, 6, 8, and 12 hours in the medium. The remaining animals were then placed in a large container of sea water before sacrifice at 22 and 31 hours from when they were first placed in the medium. It was hoped that this second series would produce much higher specific activities of label in the tissues, although uptake of label by the external parts would have to be allowed for in data analysis. These animals were subsequently refered to as the "liquid medium group."

The specimens from both groups were permitted to slowly decalcify in several changes of cold (4°C) acidic fixative over the next 7–10 days. They were then brought up to and repeatedly extracted in 70% ethanol to remove picric acid. Eventually, they were dissected into disc and arm pieces and prepared as 10 μ-thick paraffin sections. About 40 slices of serial transverse sections, taken from the bivial interbrachium to the mouth, were prepared from each animal, together with several additional slides of arm sections. These were examined, still paraffinized, and 10 to 15

containing representative regions were selected for further study. They were then deparaffinized, wrapped in Kodak AR-10 stripping film, and kept together with dessicant in dark boxes in a refrigerator until developed, 5 to 12 months later. The final autoradiographs were left unstained, and were mounted under glycerine jelly for examination.

These autoradiographic methods permit the visual localization to within a few microns of deposited, insoluble (usually proteinoid) material derived from the original labeled amino acids. Any tracer that was metabolized and excreted was, of course, lost. Thus, while positive exposure of the photosensitive emulsion indicates the definitive presence at that site of derived nutritive material, other, unexposed areas may have contained varying amounts of unregistered label in the living animal. These considerations must be carefully weighed in interpreting the results, and particular care must be exercised in quantitative judgments.

Results

Distinctive morphology

The internal structure of *Echinaster graminicolus* has been partially described in earlier reports (Ferguson, 1969, 1970) and is similar to that of related species (see Cuénot, 1887; Anderson, 1960). Distinctive is its small, eversible cardiac stomach, which connects to duct structures consisting of a series of tangential, parallel ciliated channels (Tiedemann's pouch or diverticulum) lying beneath the lower proximal part of the paired digestive glands of each arm. Aborally, each digestive gland merges into a poorly defined pyloric stomach, which, in turn, connects to a short intestine to which is attached a conspicuous pair of rectal caeca.

Photographs of autoradiographic sections of these and other parts are provided in Figures 1 to 24, arranged in time sequence. They show many newly observed anatomical details, especially of hemal tissue distribution. Clearly revealed are the small irregular, mesenteric hemal "vessels" found at the junctures of the digestive glands and their supportive paired mesenteries (Fig. 12). In the disc, these join together with other very small vessels on the stomach supportive ligaments and an aboral hemal ring embedded in the integument (Figs. 7, 23). Separate "gastric hemal tufts," reported in other species, apparently do not occur in *Echinaster*. The aboral hemal ring connects conspicously (Figs. 7, 9, 13, 14, 23) to the axial organ lying adjacent to the stone canal (Fig. 13 and others). The axial organ is composed of a histologically complex series of spongey sinuses. It is entirely contained in its own chamber (the axial sinus), which is an extension of the perihemal coelomic compartment. The wall of this sinus is rather thick in the middle (Fig. 17), but much thinner at its upper and lower ends (Figs. 9, 18, 23). No distinct connections could be seen between it and the perivisceral coelom or the water vascular vessels. Aborally, the axial organ terminates in a stubby "head" process (Figs. 9, 23), and orally it connects to the oral hemal ring (Figs. 2, 19) surrounding the mouth. From the aboral hemal ring also radiate, within perihemal channels contained in the body wall, genital hemal "connectives" to the genital hemal sinus of each gonad (Figs. 15, 16, 24). The oral hemal ring connects to five ambulacral radial hemal strands, which give off branches to a connective tissue-hemal plexus located in each tube foot (Fig. 11).

The photographs also show other characteristic features of the water vascular system. *Echinaster* has an aboral madreporite which opens into a ciliated stone canal containing a central "T"-shaped ridge (Figs. 5, 6, and others). Orally, the stone canal connects to a ring canal which medially gives off a number of spongey Tiedemann's

bodies protruding into the perivisceral coelom (Figs. 1, 2, 19). From the ring canal also extend the radial canals in the ambulacrum of each arm, which give off valvulated transverse vessels to the ampullae and tube feet (Figs. 11, 20). Beneath the irregular coelomic lining of the tube feet is a muscle layer, connective tissue-hemal plexus, and thick columnar epidermis (Fig. 11). The epidermis is highly rugose, especially at the mid level of the tube foot (Fig. 20).

Tracer distribution, solid food group

Regions of retained radioactivity were detected in the internal parts of all the specimens fed solid food, but the levels were generally low, and assessment had to be accomplished primarily by comparing grain densities of selected areas. Table I summarizes the overall interpretation of many individual observations of different sections and regions. While useful comparative differences are implied by the number of plus symbols, a truly quantitative evaluation did not seem justified. In addition to the structures shown, label was detected in irregular patches of the lining of the cardiac stomach, just inside the buccal membrane, and in the oral regions. Relatively little activity was found in the pyloric stomach or the rectal caeca, except after 12 or 24 hours.

The table shows the progressive increases in incorporation of label seen in the digestive glands throughout the 12-hour feeding period. This activity was largely localized in the mid to lower regions. Notable, but lower levels of activity were found in the Tiedemann's diverticula, mainly near the adhesive zones. The label was retained primarily in the inner third or half of the columnar lining cells of the digestive glands, somewhat inward of the lumenal border. In specimens sacrificed 24 and 48 hours after beginning feeding, the distribution was more uniform (and not as locally intense), but similar in basic pattern. These latter animals possessed a distinct zone of labeling located in the connective tissue-hemal plexus on the coelomic side of the digestive organs.

Table I also shows a progressive appearance of label in the major hemal structures. Particularly noteworthy was an observed build up by 12 hours of radioactive substance in the axial organ, and later appearances in the genital hemal connectives and genital hemal sinus.

TABLE I

Relative intensity of label in Echinaster *digestive and hemal tissues after ingestion of solid food*

Hours from start	2	4	6	8	12	24	48
Tiedemann's diverticulum	0	0	++	+	+	+	+
Mid digestive gland	0	++	+++	++++	++++	+++	++
Upper digestive gland	0	+	+	+	++	++	+
Mesenteric hemal vessel	0	0	0	++	++	+++	+++
Aboral hemal ring			0		++	++++	+++
Upper axial organ			0		++	+++	++
Lower axial organ			0		+	++	+
Genital hemal connective			0		+	0	+++
Genital hemal sinus			0		0	0	+++
Oral hemal ring			0		0	+	++
Radial hemal strand	0	0	0	0	++	+	++
Tube foot hemal plexus	0	0	0	0	+	+	+

TABLE II

Relative intensity of label in Echinaster *digestive and hemal tissues after ingestion of liquid medium*

Hours from start	3	4.5	6	8	22	31
Cardiac stomach		+++	++++	++++	+++	+++
Pyloric stomach		0	0	0	+	+
Rectal caeca	0	0	0	+	++	+++
Tiedemann diverticulum	++	+++	++	++	+++	+++
Mid digestive gland	+	++	+	+++	+++	+++
Upper digestive gland	0	+	0	+	++	++
Dig. gland hemal plexus	0	+	0	++	++++	+++
Mesenteric hemal vessel	0	0	0	+	++++	++++
Aboral hemal ring	0	0	0	++	++++	++++
Upper axial organ	0	0	+	++	++++	+++
Axial organ head process	0		0	+	+	++
Lower axial organ	0	0	+	++	++++	+++
Genital hemal connective	0	0	0	+	+++	++++
Genital hemal sinus	0	0	0	+	++++	++
Oral hemal ring		+		+	+++	++++
Radial hemal strand	0	0	0	+	+++	++++
Tube foot hemal plexus	0	0	0	0	++++	++++

Tracer distribution, liquid medium group

The animals fed liquid medium, as expected, ingested a much higher specific activity of nutrients than those of the solid food group. This permitted a more accurate visual localization of tracer in their tissues by noting relative darkening without, in most cases, resorting to counting individual grains. Table II summarizes the observed progressive appearance of label in the digestive, hemal, and reproductive systems. The Figures (1 to 24) represent examples of specific autoradiographs, arranged to show the progressive changes that occurred. These figures illustrate many details of label distribution and variability beyond those evident in the tabulated results.

Before proceeding, it first must be noted that in all specimens a large quantity of tracer must have directly entered the madreporite and become incorporated throughout the lining of the water vascular system, and in amoebocytes found in this system. Most notable was the uptake on the lateral margins of the inner ridge of the stone canal (Figs. 6, 13, and others), the lining of the ring and radial canals (Figs. 2, 11, 20), and the Tiedemann's bodies (Figs. 1, 2, 19). Lesser amounts were seen in the coelomic linings of the tube feet and ampullae (Figs. 11, 20). Labeled material did not appear to have moved through the linings of these structures into other tissues.

The major quantity of tracer taken up by the animals was, of course, ingested. After only a few hours, some label had been incorporated into the tissues of the cardiac stomach, Tiedemann's diverticula, and mid to lower portions of the digestive glands. Within the Tiedemann's diverticula, this label was initially localized near the adhesive zones which form the parallel tangential vessels (Fig. 3), and in individual cells, mainly in the upper lateral portion of the lower duct (Figs. 1, 3).

By 8 hours, the distribution of tracer in the digestive glands was a little more complete, but with concentrations still in the lower portions of the festoons, and near the base of the columnar cells (Figs. 4, 7). The adhesive zones of the Tiedemann's diverticula continued to retain the most activity, except for the immediate junctures (Fig. 4). In later animals, there were increased accumulations of radioactive material in the mid and upper digestive gland, more uniformly distributed throughout the cells (compare Figs. 7, 10, 12, 23). Another major change was the gradual build up

of evenly distributed label in the rectal caeca. By 31 hours this build up was quite marked (Fig. 21).

Prominent levels of tracer first appeared in the hemal tissues after 6 to 8 hours. At that time it was found simultaneously in the mesenteric hemal vessels, aboral hemal ring, and fairly uniformly throughout all portions of the axial organ except its very upper and lower ends (Figs. 6, 7, 9). Lesser amounts were found in the other hemal tissues, including the digestive gland hemal plexus, genital hemal connectives (Fig. 8), genital hemal sinus, oral hemal ring, and the radial hemal strands. By 22 and 31 hours, almost all these hemal areas had become very intense with label, which had also spread into the hemal plexus of the tube feet (Fig. 11), and strongly into the genital hemal sinus (Figs. 15, 16). While the hemal layers of the digestive glands showed distinct concentrations (Figs. 12, 22), as did the mesenteric hemal vessels, connections between these were rare and intermittent (Fig. 12). Some activity could be traced nearly continuously from the mesenteric hemal vessels to the aboral hemal ring, to the axial organ, and to the genital hemal connectives (Fig. 23). Much of this appeared to be contained in the fixed hemal substance rather than in cellular components.

As shown in Table II, the build up of activity in the genital hemal sinus, lower genital hemal connectives, and the connective tissue-hemal plexus of the tube feet occurred after that of the axial organ and the aboral hemal ring. Evidence for progressive build up of activity (as by flow of hemal substance) from the mesenteric hemal vessels of the digestive glands to the aboral hemal ring and the axial organ was looked for but not found; comparison of successive sections showed that the activity appears in most areas of these structures gradually and nearly simultaneously. Cells on the walls of the sinus-like chambers which contain the hemal tissues became somewhat irregularly labeled, and seemed to increase in radioactivity in a pattern similar to the hemal structures themselves (Figs. 11, 14, 15, 18). Of course, movements of dissolved labeled products by the fluids contained within these chambers were not observed directly by the methods employed.

Finally, it may be noted that one animal, sacrificed at 12 hours, apparently failed to ingest significant amounts of the tracer. Like other specimens, radioactivity was found in its water vascular vessels and Tiedemann's bodies, although at a somewhat lower level (perhaps reflective of more quiesent behavior during the feeding period). Of particular interest is the fact that only background levels could be detected in its axial organ and hemal tissues. Hyman (1955) and others have described openings between the ampullary chamber of the stone canal and the axial sinus. In the present material, no such openings were found and the levels of labeling in the upper ends of the axial organs were less than elsewhere in the organs.

Discussion

Hemal system function

Demonstration of circulatory-type functions in echinoderm hemal systems by using markers has been previously attempted by a number of workers, including Cuénot (1901), Oomen (1926), Stott (1955, 1957), Campbell (1966), Rosati (1970), and Broertjes *et al.* (1980). Since these investigators mainly relied on non-nutritive tracers, their results left many unanswered questions. Ferguson (1963a, b), tried to overcome this problem by feeding small clams injected with C^{14}-labeled nutrients to *Asterias forbesi,* and following the tracer's distribution with autoradiographic and counting methods. Activity was found retained principally in the digestive glands,

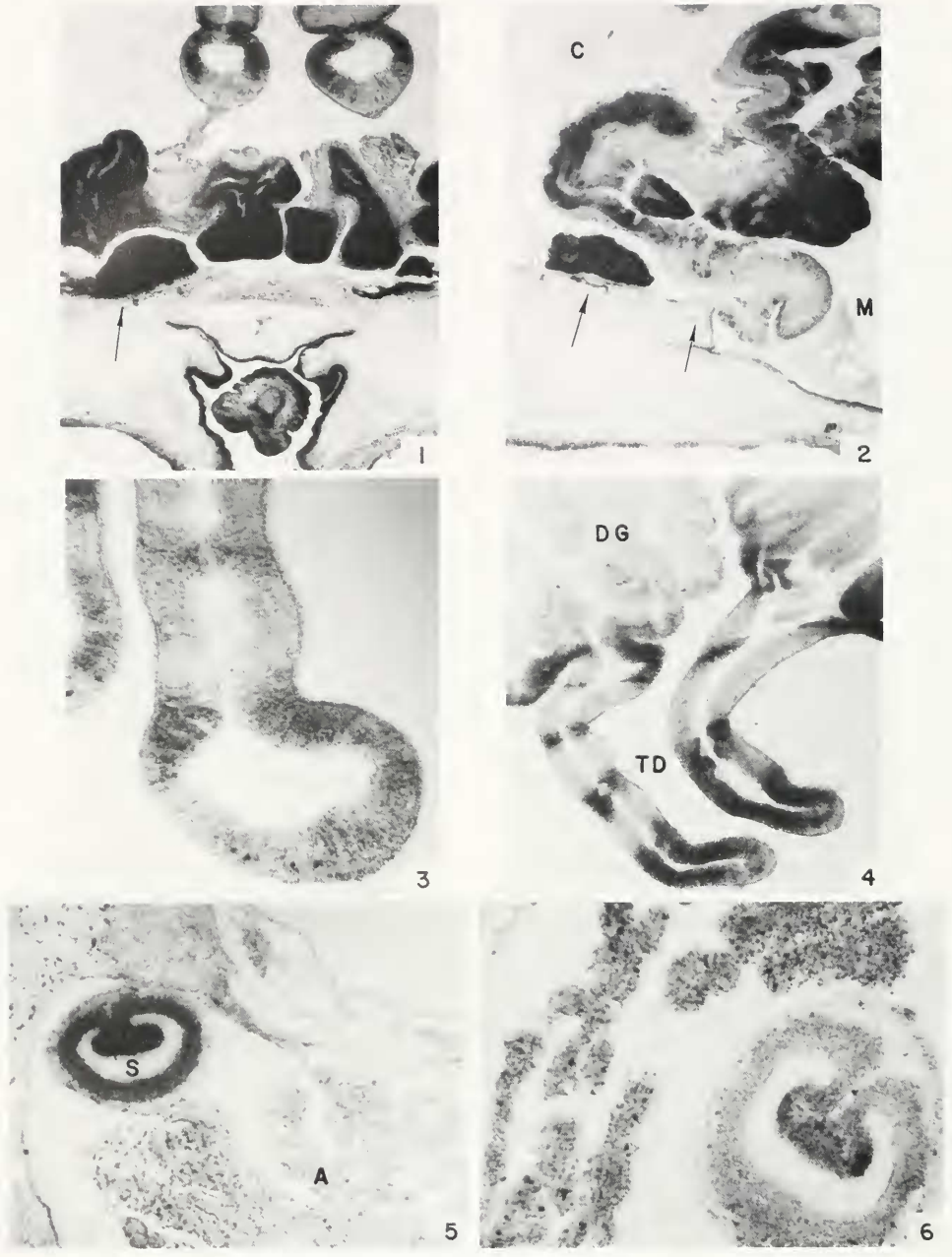


FIGURE 1. Like other figures, an unstained autoradiograph of a section through the oral disc of *Echinaster* fed labeled liquid food. Sacrificed 4.5 hours after start. Arrow shows intense activity in a Tiedemann's body, which connects to the ring canal on left. Above it are heavily labeled portions of the cardiac stomach. Above these are the lower parts of the Tiedemann's diverticula. 80×.

FIGURE 2. Near mouth region after 8 hours from start. Left arrow points to intense label in ring canal and Tiedemann's body; right arrow to weakly labeled oral hemal ring, located in perihemal sinus above nerve ring. C = coelom; M = mouth. Above mouth is cardiac stomach. 80×.

although erratic patches were noted in the hemal septum and other tissues. In light of the dominance of coelomic translocative mechanisms, the importance of the hemal system was discounted. In later experiments, however, stronger evidence was found that the hemal system was involved in nutrient translocation. Labeled amino acid injected into the body cavity of *Echinaster* was located intermittently in parts of the radial hemal strand and the connective tissue-hemal plexus of the tube feet.

The present study provides a much more complete picture of nutrient translocation through the hemal system. It demonstrates that within a few hours of their ingestion, labeled nutrients preferentially begin to appear in the axial organ, and at a somewhat slower rate, in the ambulacral radial hemal strands and other hemal structures. Retention is primarily in the form of non-cellular hemal substance, which after fixation is insoluble in the normal histological solvents. The origin of this material is still unknown, but its nearly simultaneous appearance in multiple locations suggests that it may be initially dispersed through the coelom.

Labeled hemal material appears to move from the radial hemal strand to the connective tissue-hemal plexus of the tube feet, and on to their distal ends where mucus glands are located (Fig. 11). The question of whether this material supports muscular activity in the tube foot wall could not be answered by the autoradiographic method. It was observed that the thick rugose epidermis of the tube feet have an extraordinary affinity for the labeled liquid medium. This propensity for uptake, coupled with the fact that echinoderms possessing particularly active tube feet have an unusually high metabolic dependence on dissolved exogenous nutrients (Ferguson, 1982c), suggests that tube feet depend heavily on external nutrient sources. The tube foot muscles, however, are located inward of the connective tissue-hemal plexus. While it is possible that nutrients are passed from the epidermis to the hemal tissue, some labeled material was found within the tube feet of animals fed solid food (Table I).

Subsequent to the appearance of label in the axial organ, labeled material was found in the genital hemal connectives and then the genital hemal sinus itself. This is an entirely new observation and it conforms to the hypothesis recently suggested by Walker (1980), that the gonads depend on the hemal system for the collection and storage of nutrients used in gametogensis. It is somewhat surprising that the pattern of movement of labeled material towards the gonads is as obvious as it is considering that only a few weeks earlier the experimental animals had completed spawning. No differences were seen between the males and females.

While it now can be concluded that the starfish hemal system, along with possible other functions, does indeed play a significant role in the distribution of nutritive material within the body, the present study cannot fully explain how the system functions in such translocation. Some additional insights gleaned from the observations, however, may be helpful.

It is possible that nutrients are collected in the hemal layer of the digestive glands, are passed up to the mesenteric hemal vessel, and then pass centrally to junctions

---

FIGURE 3. Lower Tiedemann's diverticulum, 4.5 hours after start. Maximum label is in cells near adhesive zones and in lower duct. 220×.

FIGURE 4. Tiedemann's diverticula (TD) attached to digestive glands (DG), 8 hours after start. Maximum activity is in lower duct, adhesive zones, and lower digestive gland. 80×.

FIGURE 5. Stone canal (S) and axial organ (A), 4.5 hours after start. Intense label is in stone canal, especially on central ridge. Most darkening of axial organ and other tissues is here not label, but natural pigmentation. Aboral hemal ring joins at upper right. 80×.

FIGURE 6. Stone canal and axial organ after 8 hours. Most activity is in lateral regions of inner ridge of stone canal. Moderate activity (and pigment) is now located throughout axial organ. 275×.

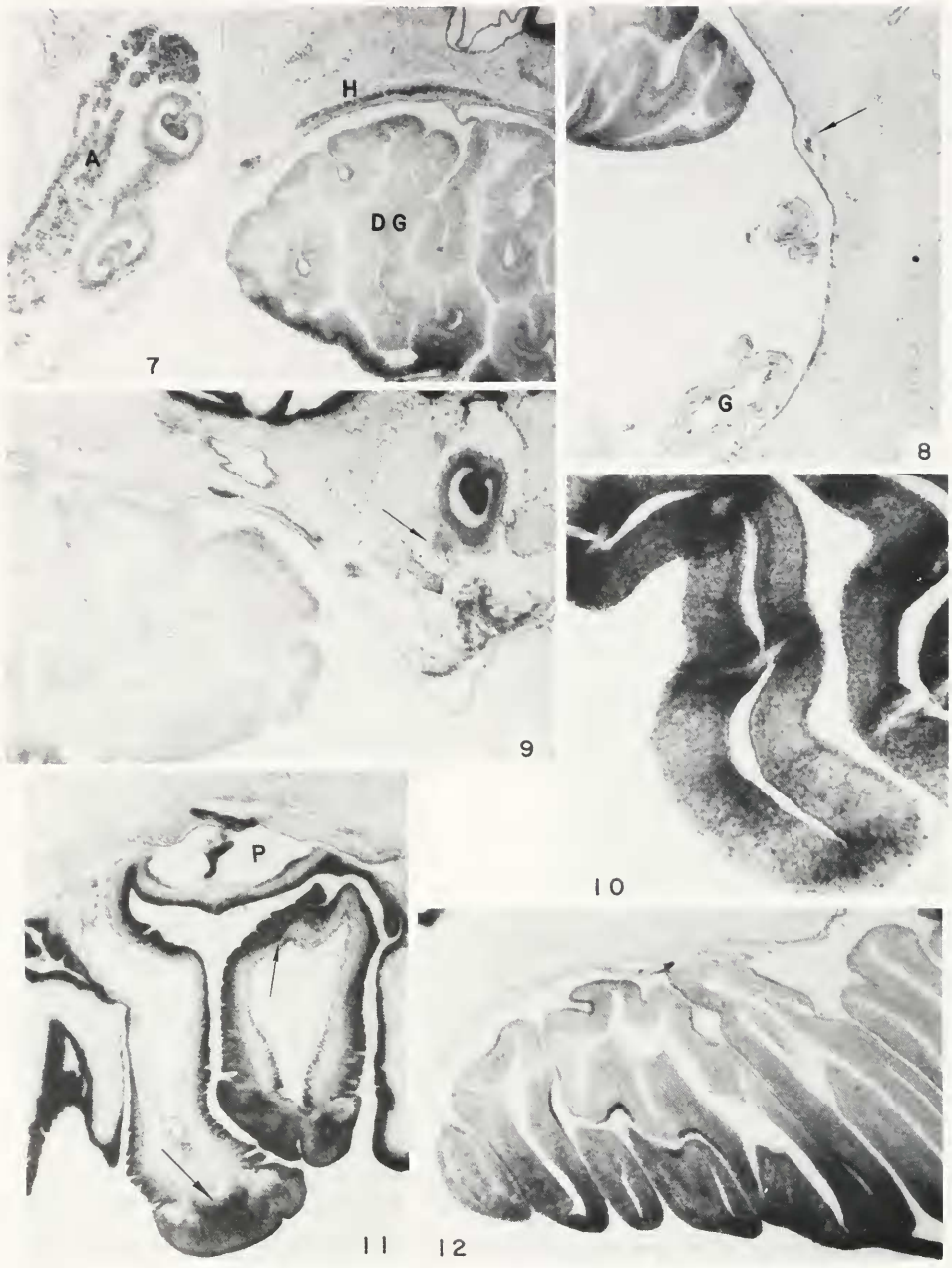


FIGURE 7. After 8 hours. Axial organ (A) and stone canal may be seen within the axial sinus, on left. Aboral hemal ring (H) extends above digestive gland (DG); both it and axial organ contain moderate label. Little label is in upper digestive gland and its supporting mesentery. 80×.

FIGURE 8. After 8 hours. Arrow indicates moderate label in genital hemal connective extending to as yet unlabeled gonad (G). 80×.

FIGURE 9. After 8 hours. A section more central than that of Figure 7. Shows highly labeled upper portion of stone canal near its connection to the madreporite. Arrow points to part of lightly labeled axial organ head process. Edge of the moderately labeled axial organ proper is below it, connecting to aboral

with the aboral hemal ring and the axial organ. Cuénot (1887, 1891, 1948) has described such vessels, and connections between these parts were seen in the present material. Two facts, however, cast doubt on such a interpretation. First, the "vessels" involved are diminuitive and appear incapable of translocating very much material. Indeed, the digestive gland hemal plexus is poorly developed near the mesenteries (Figs. 7, 9, 12). Second, the build up of tracer within these mesenteric vessels and most parts of the axial complex occurs nearly simultaneously. Perhaps a progression was missed between the time intervals selected for study, but that was not the impression gained through careful study of this point with many slides. As noted, a definite progression of label down the genital hemal connectives to the genital hemal sinus was observed.

Earlier workers, dating back to Cuénot (1887, 1901), Chapeaux (1893), and Cohnheim (1901), had developed an alternative hypothesis, that nutrient translocation could be accomplished by migration of amoebocytes. Labeled amoebocytes were searched for in the autoradiographs. Since only a few were seen, mainly in the water vascular system and axial sinus, their significant contribution to the translocative function and the production of hemal substance also can be ruled out.

Broertjes *et al.* (1979), working with *Asterias rubens,* believed that they had found evidence that nutrients were transported centrally in mucus along the inner aboral surface of the digestive glands to the aboral pyloric stomach, and then, by way of the gastric hemal tufts, to the axial organ. In the present study, little tracer was found in the aboral part of the digestive gland or in the pyloric stomach, and structures readily identifiable as gastric hemal tufts did not exist (they may be more characteristic of larger species). The mesenteric hemal vessels appear to join directly with the aboral hemal ring (Fig. 7), with only minor extensions on ligaments extending to the pyloric stomach. While the latter are found to contain varying amounts of tracer, they do not appear to be major conduits.

The extensive accumulation of tracer throughout the axial organ, becoming particularly apparent 6 to 12 hours after feeding, suggests that this organ concentrates nutrients reaching it in a fluid state via the coelomic spaces. The digestive glands carry on nutrient exchanges with the circulating fluids of the perivisceral coelom (Ferguson, 1964a, b). While direct connections between the perivisceral coelom and the axial sinus and other perihemal channels could not be found, there are areas where the two compartments are separated only by the thinnest of membranes. These include the upper and lower end of the axial sinus (Figs. 18, 23), and interpodially along the floor of each arm (Ferguson, 1970). Further, the spongey structure of the axial organ (see Bargmann and von Hehn, 1968; Leclerc, 1974) is appropriate for extraction of dissolved metabolites from the surrounding fluid. There was some indication in a few of the sections studied that the distribution of label was more intense

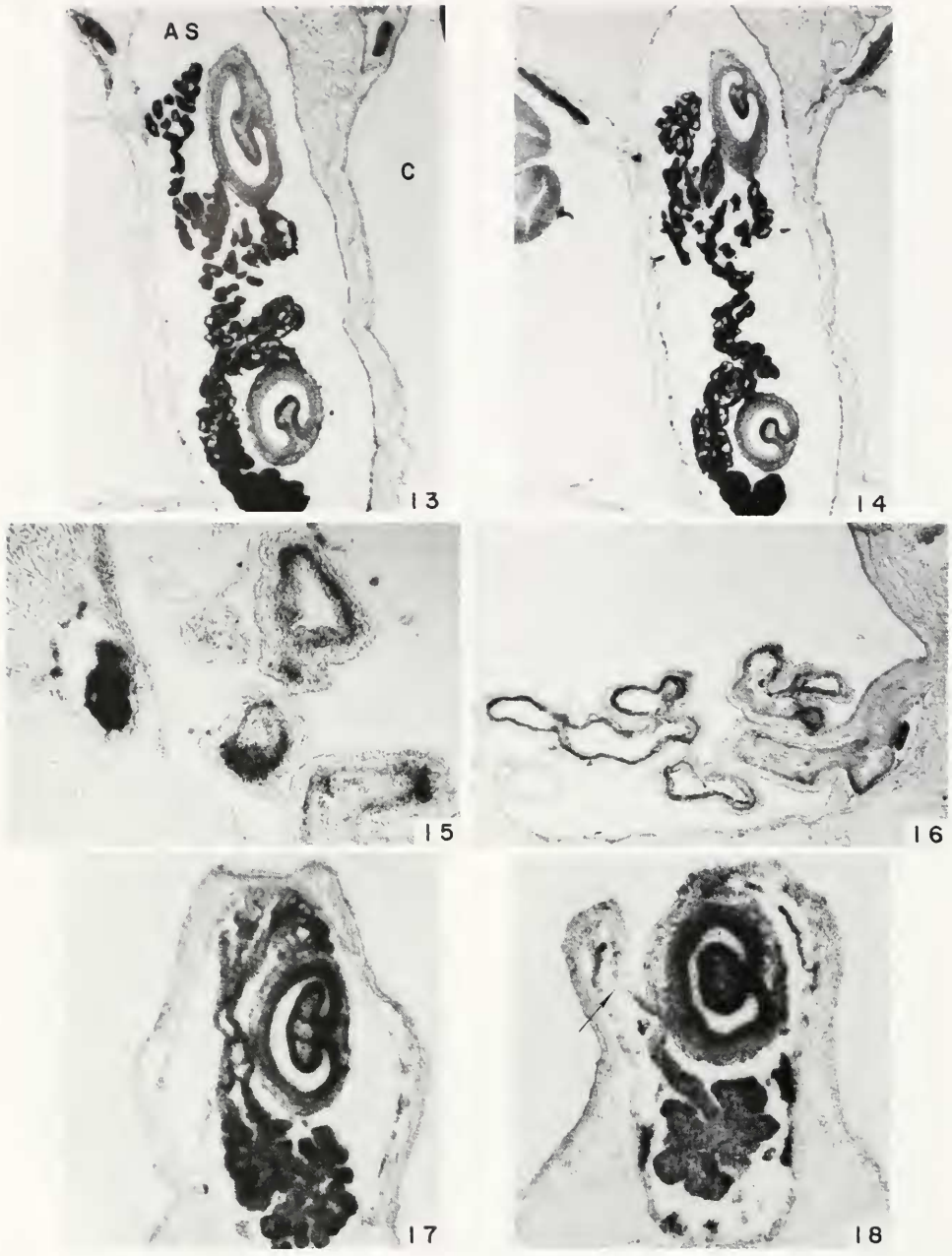
---

hemal ring extending to left. Note that there is considerably more label in this structure than in the upper digestive gland (left) and its supporting mesentery. 80×.

FIGURE 10. Tiedemann's diverticulum after 22 hours. Maximum label is still near adhesive zones, although distribution is now more general, with some intensification in outer connective tissue-hemal layer. 200×.

FIGURE 11. Ambulacral area of arm near attachment to disc, after 22 hours. Labeled radial water canal is at top, above radial hemal strand, located in perihemal sinus (P), above radial nerve cord. Arrows point to labeled hemal tissues in tube feet; these in other sections connect to the radial hemal strand. Note also, label in nerve tissue bordering the perihemal sinus. 100×.

FIGURE 12. Digestive gland and mesentery after 22 hours. Most label remains in lower part of digestive gland, but very conspicuous amounts have now moved into the connective tissue-hemal layer. One mesenteric hemal vessel is also intensely labeled. 80×.



FIGURES 13 and 14. Sections through two portions of axial sinus (AS) after 22 hours. Heavily labeled axial organ and its upper lateral connecting aboral hemal ring are plainly visible. Lateral walls of axial sinus are thick, separating it from perivisceral coelom (C). 90× and 80×.

FIGURE 15. After 22 hours. Shows intense label now in both genital hemal connective (left) and genital hemal sinus of gonad (center). 220×.

FIGURE 16. Section through another gonad at 22 hours. Genital hemal sinus is very radioactive. Gonoduct extends to right. 90×.

FIGURES 17 and 18. Transverse sections at different levels through axial sinus after 31 hours. Figure 17 is near mid portion; Figure 18 at lower end. Arrow shows thin wall at this point. Note label on inner sinus wall and periphery of axial organ. 135× and 200×.

on the exterior of the axial organ than in the interior (Figs. 17, 18, 23), but this is not certain. Labeled material was seen on the walls of the axial sinus and other perihemal spaces, also suggesting that it was probably present in the fluid.

Even though labeled hemal substances was first seen building up in the axial organ, and then progressively in the genital hemal connectives and genital hemal sinus, the mechanism of transport is uncertain. Detailed study of the hemal tissues shows a structure of congealed material contained in thin-walled, mostly acellular sinuses. While the hemal vessels seem unsuited for axial flow, the adjacent perihemal spaces, by contrast, are lined with flagellated cells oriented to create an efficient local circulation (Walker, 1979, 1980). If label is accumulated within the hemal vessels from the surrounding perihemal fluids, and is unable to flow significant distances, where might it go? An obvious possibility is that much of it could move back into the perihemal fluid for further transport. The hemal material might thus act as a local storage reservoir, while the bulk of the actual transport is accomplished by the perihemal circulation.

Would there be any functional advantage to such a complex system? This can only be determined through further study, but it is possible. A system of this kind could maintain a low concentration of metabolites in the fluids actually circulating, consistent with the levels in other compartments and osmotic requirements. This low metabolite level, in turn, would be compensated for by the very high concentration contained in the hemal substance. Continuous exchange between the perihemal fluid and the hemal material could produce a net transport. The process may be very roughly analogous to the "facilitated diffusion" mechanism by which myoglobin has been described to enhance oxygen transport in muscle tissue (Wittenberg, 1970). In any case, it is now certain that nutritive metabolites are somehow translocated within the hemal-perihemal complex.

Digestive system

The autoradiographic sections also revealed a number of properties of the digestive system, confirming in some cases conclusions drawn from previous histological studies, and in others, providing new insights. A considerable amount of nutritive material was quickly taken up by the epithelium of the cardiac stomach and the Tiedemann's diverticula (Figs. 1 to 4), showing that the cellular activity of these parts is directly supported by ingested products. The cells located near the adhesive zones and the lower tube of the Tiedemann's diverticum, and in the lower portion of the digestive gland (Figs. 3, 4), must be particularly active. Later, the label in these areas becomes more diffuse, and a new band of intensification develops near the basal connective tissue (Figs. 10, 12, 22, 23).

Interestingly, tracer is slow to move into the upper digestive gland near the mesenteric attachments (Figs. 7, 9). This region is believed to be primarily secretive and cytopoietic as opposed to absorptive (Anderson, 1960; Ferguson, 1966; de Mik-van der Plas, 1981). Only in later specimens (Fig. 23) does a more general distribution of label build up in this location. Since this buildup occurs well after the initial appearance of label in the mesenteric hemal vessels (Fig. 12), these vessels may serve primarily as reservoirs for the nutritional and trophic needs of their adjacent secretory tissues, rather than as conveyors of hemal material to other parts of the body.

The gradual build up of label in the rectal caeca (Table II) is most notable. Possibly these organs serve to remove nutritive material from the digestive contents before they are vented to the exterior. (Fluid and feces are voided by *Echinaster* at frequent intervals.) The described histological structure of the rectal caeca is well suited for such a role (Bouillon and Jangoux, 1970; Jangoux *et al.*, 1975). As there are few

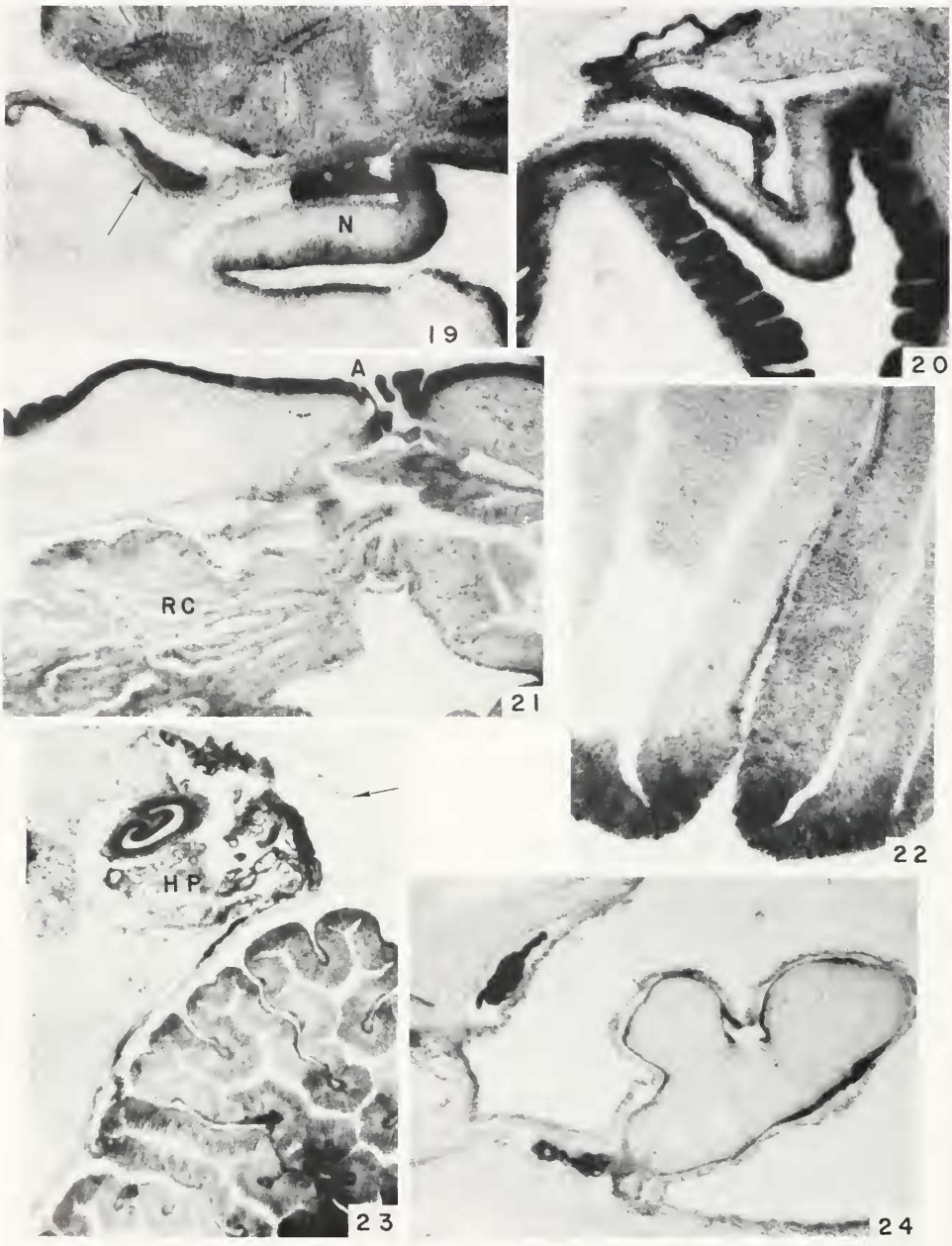


FIGURE 19. Near mouth after 31 hours. Compare to Figure 2. Arrow points to edge of Tiedemann's body. Hemal ring, above nerve ring (N), is now very heavily labeled. 200×.

FIGURE 20. Ambulacral area after 31 hours. Labeled radial hemal strands may be seen in perihemal sinus above radial nerve cord and beneath radial water canal. Lateral extensions of radial hemal strand connect with connective tissue-hemal layers of tube feet. Rugose epidermis of tube feet and lower radial nerve cord are heavily labeled by direct uptake from exterior. 260×.

FIGURE 21. Rectal caecum (RC) and anus (A) after 31 hours. A moderate level of radioactivity now exists throughout rectal caecum, whereas in earlier specimens it did not. Note that hemal tissues are not conspicuous. Epidermis has taken up label directly from medium. 80×.

attached hemal vessels on these or adjacent structures, most of the scavenged nutrients eventually may be released into the coelomic fluid circulation.

Water vascular system

Except as already noted, label could not be detected in the water vascular system of the solid food group specimens, but was very evident in those of the liquid medium group. In previous studies practically no tracer was seen entering the madreporite nor penetrating to the stone canal or deeper layers of the water vascular system. Ferguson (1967) suggested that this lack of penetration was due to the efficiency of uptake by the outer layers, thereby preventing substances (at normal concentrations) from moving further into the system. An alternative hypothesis had been argued by Binyon (1964, 1966, 1976) and also supported by Prush and Whorisky (1976)—that little water enters the madreporite and that water vascular fluid replacement is accomplished osmotically.

A major difference in the present experiments from previous ones was the large amount of non-radioactive "carrier" used in the nutritive media to induce the feeding reaction. This carrier appears to have "flooded" the transport mechanism of the madreporite epidermal cells permitting the tracer to move rapidly into the deeper vessels. This observation clearly confirms that there is a significant inflow of sea water through the madreporite and into the major parts of the system. The osmotic mechanisms described by Binyon must function secondarily to this inflow.

Also of great interest was the intensive and rapid accumulation of tracer by the Tiedemann's bodies, located on the ring canal (Figs. 1, 2, 19). These organs consist of numerous radiating chambers of flagellated, cuboidal epithelia (Bargmann and Behrens, 1964). Tiedmann (1816) speculated that they filter water from the water vascular system, but later investigators have proposed a number of alternative hypotheses. Hoffmann (1875), particularly, suggested that they function in amoebocyte formation, and this view was further promulgated by Cuénot (1887, 1901) in his earlier work, and others. The absence of elevated cytopoietic activity, however, was demonstrated by Ferguson (1966), who concluded that the organs were specialized for absorption and phagocytosis, and thus served to filter body fluids.

The absorptive properties of the Tiedemann's bodies are very evident in the present work. Sea water taken up through the madreporite must be propelled down through the stone canal and ring canal, and into these structures. As they are made up of blind-ended chambers, a portion of this flow likely passes through them into the perivisceral coelom. Thus, their role now may be seen not only as puritive, but also as probably generative of the perivisceral coelomic fluid itself. This would help to explain the considerable similarity between echinoderm coelomic fluid, water vascular fluid, and sea water (see Binyon, 1966). It would also explain how starfish are able to maintain a turgid composure, especially while protruding and inflating their cardiac stomach during feeding. (Note that the one animal that did not feed had

---

FIGURE 22. Digestive gland after 31 hours. While label is more generalized than earlier, much activity is still retained in lower portions. Note intensification in connective tissue-hemal layer. 200×.

FIGURE 23. After 31 hours. Shows upper stone canal, axial organ head process (HP), edge of axial organ, and aboral hemal ring extending above a more generally labeled digestive gland. Arrow points to thin wall of axial sinus in this region. 80×.

FIGURE 24. Genital hemal connective and gonad after 31 hours. Intense label can be seen in the hemal tissues. Gonoduct extends to left. 95×.

lower levels of tracer in its Tiedemann's bodies than did the others.) Certainly, osmotic and other hydrodynamic mechanisms must at times also contribute to fluid replacement, but the importance of the Tiedemann's bodies in this function appears to have been overlooked by modern workers.

LITERATURE CITED

ANDERSON, J. M. 1960. Histological studies on the digestive system of a starfish, *Henricia,* with notes on Tiedemann's pouches in starfishes. *Biol. Bull.* **119:** 371–398.

BARGMANN, W., AND B. BEHRENS. 1964. Über die Tiedemannschen organe des Seesterns (*Asterias rubens*). *Z. Zellforsch.* **84:** 120–133.

BARGMANN, W., AND G. VON HEHN. 1968. Über das Axialorgan ("mysterious gland") von *Asterias rubens* L. *Z. Zellforsch.* **88:** 262–277.

BINYON, J. 1964. On the mode of functioning of the water vascular system of *Asterias rubens* L. *J. Mar. Biol. Assoc. U. K.* **44:** 577–588.

BINYON, J. 1966. Salinity tolerance and ionic regulation. Pp. 359–377 in *Physiology of Echinodermata,* R. A. Boolootian ed. Interscience Publ., New York.

BINYON, J. 1976. The permeability of the asteroid podial wall to water and potassium ions. *J. Mar. Biol. Assoc. U. K.* **56:** 639–647.

BOUILLON, J., AND M. J. JANGOUX. 1970. Anatomie, histologie et histochimie des caecums rectaux d'*Asterias rubens* L. *Cah. Biol. Mar.* **11:** 259–257.

BROERTJES, J. J. S., G. POSTHUMA, F. B., BEIJNINK, AND P. A. VOOGT. 1979. The admission of nutrients from the digestive system into the haemal channels in the sea-star *Asterias rubens* (L.). *J. Mar. Biol. Assoc. U. K.* **60:** 883–890.

BROERTJES, J. J. S., G. POSTHUMA, P. DEN BREEJEN, AND P. A. VOOGT. 1980. Evidence of an alternative transport route for the use of vitellogenesis in the seastar *Asterias rubens* (L.). *J. Mar. Biol. Assoc. U. K.* **60:** 157–162.

BUDINGTON, R. A. 1942. The ciliary-transport system of *Asterias forbesi. Biol. Bull.* **83:** 438–450.

CAMPBELL, J. L. 1966. *The Haemal and Digestive Systems of the Purple Sea Urchin. Strongylocentrotus purpuratus (Stimpson).* Doctoral dissertation, University of California, Los Angeles.

CAMPBELL, D. B., AND R. L. TURNER. 1984. *Echinaster graminicolus,* new species of spinusid sea star (Echinoderata: Asteroidea) from the west coast of Florida. *Proc. Biol. Soc. Wash.* (in press).

CHAPEAUX, M. 1893. Sur la nutrition des échinodermes. *Bull. Acad. R. Belg.* **26:** 227–232.

COHNHEIM, O. 1901. Versuche über resorption, verdauung und stoffwechseln von echinodermen. *Z. Physiol. Chem.* **33:** 9–54.

CUÉNOT, L. 1887. Contribution a l'étude anatomique des astérides. *Arch. Zool Exp. Gen.* **5**(suppl.): 1–144.

CUÉNOT, L. 1891. Études morphologiques sur les échinodermes. *Arch. Biol.* **11:** 313–680.

CUÉNOT, L. 1901. Études physiologiques sur les astéries. *Arch. Zool. Exp. Gen.* **9:** 233–259.

CUÉNOT, L. 1948. Anatomie, éthologie et systématique des echinodermes. Pp. 3–363 in *Traite de Zoologie,* Vol. XI, P. P. Grassé, ed. Masson, Paris.

DE MIK-VAN DER PLAS, A. 1981. *Pyloric Caeca of the Starfish Asterias rubens L. During the Reproductive Cycle: A Histological and Physiological Study.* Doctoral dissertation, University of Utrecht.

FERGUSON, J. C. 1963a. *The Physiological Mechanisms of Nutrient Transport in the Starfish,* Asterias forbesi. Doctoral dissertation, Cornell University.

FERGUSON, J. C. 1963b. An autoradiographic study of the distribution of ingested nutrients in the starfish, *Asterias forbesi. Am. Zool.* **3:** 524.

FERGUSON, J. C. 1964a. Nutrient transport in starfish. I. Properties of the coelomic fluid. *Biol. Bull.* **126:** 33–53.

FERGUSON, J. C. 1964b. Nutrient transport in starfish. II. Uptake of nutrients by isolated organs. *Biol. Bull.* **126:** 391–406.

FERGUSON, J. C. 1966. Cell production in the Tiedemann bodies and hemal organs of the starfish, *Asterias forbesi. Trans. Am. Microsc. Soc.* **85:** 200–209.

FERGUSON, J. C. 1967. An autoradiographic study of the utilization of free exogenous amino acids by starfishes. *Biol. Bull.* **133:** 317–329.

FERGUSON, J. C. 1969. Feeding activity in *Echinaster* and its induction with dissolved nutrients. *Biol. Bull.* **136:** 374–384.

FERGUSON, J. C. 1970. An autoradiographic study of the translocation and utilization of amino acids by starfish. *Biol. Bull.* **138:** 14–25.

FERGUSON, J. C. 1982a. A comparative study of the net metabolic benefits derived from the uptake and release of free amino acids by marine invertebrates. *Biol. Bull.* **162:** 1–17.

FERGUSON, J. C. 1982b. Nutrient translocation. Pp. 373–393 in *Echinoderm Nutrition,* M. Jangoux and J. M. Lawrence, eds. Balkema, Rotterdam.

FERGUSON, J. C. 1982c. Support of metabolism of superficial structures through direct net uptake of dissolved primary amines in echinoderms. Pp. 345–351 in *Echinoderms: Proceedings of the International Conference, Tampa Bay,* J. M. Lawrence, ed. Balkema, Rotterdam.

HOFFMANN, C. K., 1875. Zur anatomie der asteriden. *Niederl. Arch. Zool.* **2:** 1–32.

HYMAN, L. H. 1955. *The Invertebrates: Vol. IV, Echinodermata.* McGraw-Hill, New York.

JANGOUX, M., C. MASSIN, AND E. VAN IMPE. 1975. Mise en evidence du rôle émonctoire des rectaux d'*Asterias rubens* L. (Echinodermata, Asteroidea). *C. R. Acad. Sci. Paris Ser. D* **281:** 643–646.

LECLERC, M. 1974. L'organe axial et ses relations avec la sexulaité et l'immunité chez les astérides. *Ann. Sci. Nat. Zool. Paris* **16:** 285–360.

PRUSH, R. O., AND F. WHORISKY. 1976. Maintenance of fluid volume in starfish water vascular system. *Nature* **262:** 577–578.

ROSATI, F. 1970. The fine structure of the alimentary canal of holothurians. 2. The uptake of ferritin and iron-dextran. *Monit. Zool. Ital.* **4:** 107–113.

OOMEN, H. A. 1926. Verdauungsphysiologische studien an Holothurien. *Pubbl. Stan. Zool. Napoli* **7:** 215–297.

STOTT, F. C. 1955. The food canal of the sea-urchin *Echinus esculentus* and its functions. *Proc. Zool. Soc. Lond.* **125:** 63–86.

STOTT, F. C. 1957. Observations on the food canal and associated structures in the holothurian *Holothuria forskali. Proc. Zool. Soc. Lond.* **129:** 129–136.

TIEDEMANN, F. 1816. *Anatomie der Rohren-Holothurie, des Pomeranzfarbigen Seesterns und Stein-Seegels.* Thomann, Landshut.

WALKER, C. W. 1979. Ultrastructure of the somatic portion of the gonads in asteroids, with emphasis on flagellated-collar cells and nutrient transport. *J. Morphol.* **162:** 127–162.

WALKER, C. W. 1980. Spermatogenic columns, somatic cells and the microenvironment of germinal cells in the testes of asteroids. *J. Morphol.* **166:** 81–107.

WITTENBERG, J. E. 1970. Myoglobin-facilitated oxygen diffusion: Role of myoglobin in oxygen entry into muscle. *Physiol. Rev.* **50:** 559–636.